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Preventing tick attachment to dogs using essential oils

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ABSTRACT

Preventing tick bites using repellents could make a valuable contribution to an integrated tick management programme for dogs. Here, the ability of a range of essential oils or active ingredients of commercially available repellents, to abolish the orientation and taxis of the tick *Ixodes ricinus* towards sebum extracted from dog hair was examined in laboratory bioassays. Substantial differences between oils were observed, but turmeric oil was both able to prevent a climbing response by ticks and had a longer residual activity than other oils. A blanket-drag field assay was then used to compare the attachment of ticks to blankets impregnated with one of: turmeric oil, DEET (positive control), orange oil or excipient only (negative controls). In total, 899 ticks were counted, with an average of 23.3 (SD±21.3) ticks per blanket drag for excipient-only (n = 16), 26.9 (SD±28.6) for orange oil (n = 16), 2.6 (SD±2.0) for turmeric oil (n = 16) and 3.4 (SD±3.7) for DEET (n = 16). Finally, in a participatory *in vivo* trial, tick acquisition by 15 untreated control dogs was compared with 24 dogs sprayed with turmeric oil and 16 dogs sprayed with orange oil (both 2.5 % v/v diluted in water with a 1% coco glucoside excipient) before each walk in known tick infested areas. The percentage of dogs with ticks attached to the legs or belly of dogs sprayed with turmeric oil suspension (15% ±19.4%) was significantly lower than that of ticks attached to the same areas of dogs sprayed with orange oil suspension (85% ±19.4%) and unsprayed dogs (73% ±26.2%) ($P<0.05$). The data indicate that turmeric oil may form a valuable component of a tick management programme for domestic dogs.

Key words: tick, *Ixodes ricinus*, dog, treatment, repellence, attachment, essential oil

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1. Introduction

Effective control and management of ticks may best be achieved using a multifaceted approach; combining the benefits of a range of methods is likely to increase probability that ticks and tick-borne pathogens are more effectively eliminated. With neurotoxic acaricides, efficacy and residual activity depend on the active ingredient and mode of application, with the range of topical, systemic or slow-release products currently available offering a mixture of advantages and disadvantages. This has encouraged the commercialisation of combinations of actives, offering different complementary properties, and a search for alternative methods of tick control, such as vaccines and bio-control with parasitoids, predators and entomopathogenic fungi (Samish et al., 2008; Perez-Perez et al., 2010). As part of a tick management programme, avoidance and prevention of tick bites, using repellents, may also make a valuable contribution (Ellse & Wall, 2013; Lupi et al., 2013; Abdel-Ghaffar et al., 2015).

Two types of repellency are defined (Halos et al., 2012). The first, repellency *sensu stricto*, may be attributed to a compound producing an irritant effect through direct contact, which causes a tick to move away from the treated surface/animal or to fall off before attaching to the host. The latter, repellency *sensu lato* (or expellency), causes the inhibition of attachment or the detachment of already attached ticks. In the last decade, there has been extensive research into the repellent effects of many compounds against ticks. The majority of these studies have focused on *in vitro* studies of *sensu stricto* repellence (Pamo et al., 2005; Ribeiro et al., 2008; Cetin et al., 2010; Štefanidesová et al., 2017).

A variety of commercial tick repellents are available, including both synthetic and plant derived compounds (Nerio et al., 2010; Lupi et al., 2013; Rehman et al., 2014), including DEET (N,N-Diethyl-3-methylbenzamide), IR3535 (3-N-acetyl-N-butylamino-propionic ethyl ester), icaridin (1-piperidine-carboxylic acid 2e2 hydroxyethyl-1-methylester), as well as a natural *Eucalyptus citriodora* derivative (para-menthane-3,8-diol) (Semmler et al., 2009; Abdel-Ghaffar et al., 2015; Benelli et al., 2016).

Plant-derived essential oils are blends of approximately 20–80 different metabolites which are usually extracted from plants via steam distillation (Bakkalai et al., 2008). These metabolites are volatile molecules of low molecular weight and usually contain two or three major terpene or terpenoid components, which constitute up to 30% of the oil (Bakkalai et al., 2008). There is a growing body of evidence indicating that they possess varying

mechanisms of action against arthropods; they have been shown to inhibit feeding and the synthesis of chitin, decrease growth, development or reproduction, and affect behaviour including acting as repellents (Pazinato et al., 2016; Rosado-Aguilar et al., 2017). The efficacy of essential oils is often attributed to the oil's major component(s); however, there is also evidence that the various oil components may work in synergy (Nerio et al., 2010). This may occur because some oil components aid cellular accumulation and absorption of other toxic components (Cal, 2006).

The efficacy of essential oils against ticks has been demonstrated following immersion and physical contact with treated surfaces, as well as after exposure to the vapour of oils; the latter implies that there is a neurotoxic, rather than simply a mechanical pathway in their mode of action. Terpinen-4-ol, for example, a monoterpene found at high concentrations in tea tree oil, inhibits arthropod acetylcholinesterase, an enzyme essential for transmission of action potentials (Mills et al., 2004; Lopez & Pascual-Villalobos, 2010). Additionally, the hydrophobic nature of the oils may simultaneously exert mechanical effects on the parasite such as by disrupting the cuticular waxes and blocking the spiracles, which leads to death by water stress or suffocation (Burgess, 2009).

The aims of the work presented here were to use an *in vitro* laboratory bioassay to screen essential oils for repellency in the tick *Ixodes ricinus*, to further test the most promising oils using a blanket-drag field assay and finally to investigate the efficacy of oils as natural tick repellent for dogs walked regularly in tick infested areas.

2. Materials and methods

2.1 Repellency bioassay

The oils used in this study were selected based on previous reports of biological activity. Essential oils from bog myrtle (*Myrica gale*), cajeput (*Melaleuca cajeputi*), geranium (*Pelargonium graveolens*), ginger (*Zingiber officinale*), grapefruit (*Citrus paradisi*), lavender (*Lavendula angustifolia*), niaouli (*Melaleuca viridiflora*), orange (*Citrus sinensis*), peppermint (*Mentha arvensis*), spearmint (*Mentha spicata*), thyme (*Thymus vulgaris*) and turmeric root (*Curcuma longa*) were used, as well as the carrier oils blackseed (*Nigella sativa*) and soya (*Soja hispida*). The oils were screened initially at a concentration 5% (v/v), following previously published studies (Ellse & Wall, 2013). All essential oils were obtained from one supplier (Naissance Trading & Innovation Co Ltd., Neath, United Kingdom) and

had been extracted via steam distillation, with the exception of the citrus peel oils (orange and grapefruit), which had been cold pressed. Oils were stored at 4°C to prevent thermodegradation or oxidation. Ethanol (Sigma-Aldrich, ≥99.8%, Scientific Laboratory Supplies Ltd., Dorset, United Kingdom) was used to dilute the oils to varying concentrations. As an attractant, sebaceous secretions were extracted from hair clippings obtained from an English Springer Spaniel (Crooks and Randolph, 2006). Clumps of hair were chopped, placed in 50 ml of methanol (Sigma-Aldrich, 99.8%, Scientific Laboratory Supplies Ltd., Dorset, United Kingdom) and stirred continuously for 10 min. The beaker was then allowed to stand at room temperature ($21 \pm 1^\circ\text{C}$) for 48 h and the remaining liquid strained from the hair using a sieve. The sebum suspension was divided into aliquots and stored at -20°C until use. Ethanol only and a suspension of olive oil in ethanol, provided negative controls (Martinez-Velazquez et al., 2011). DEET (N,N-Diethyl-3-methylbenzamide) and PMD (p-menthane- 3,8-diol, Sigma-Aldrich, Scientific Laboratory Supplies Ltd., Dorset, United Kingdom, which is found in small quantities in the essential oil from the leaves of the *Eucalyptus citriodora* tree and used as an active ingredient in many insect repellents, provided positive controls.

Filter paper strips (8 x 1 cm) were suspended from one end by a cotton thread. Before each test, 10 µl of test compound was applied to the end of the filter paper strip from which it was suspended using a pipette; this amount was sufficient to impregnate the top 1 cm of the filter paper only. The ethanol was allowed to evaporate prior to testing (≈ 30 s). The strip was suspended vertically, 1 cm below the tip of a horizontal glass rod. 50 µl of the dog hair sebum attractant was applied to a circle of filter paper (15 mm diameter), which was then allowed to dry for 5 min in a fume cupboard. This was attached to the tip of the glass rod, using double sided tape, so that it was directly above the suspended vertical strip of filter paper (Fig. 1).

Nymphal *I. ricinus* were collected using a standardised blanket-dragging technique from vegetation at the edge of an area of woodland in south west England. After collection, nymphs were stored at 7°C and were acclimated to room temperature ($21 \pm 1^\circ\text{C}$) for 24 h prior to testing. Ticks were used within three days after collection and each tick was used only once. A fine paintbrush was used to transfer an individual tick, selected at random, onto the centre of the suspended vertical strip of filter paper and its behaviour was observed for 5 min, to record the movement of the tick on the filter paper. The number of ticks that reached the top of the filter paper strip or dropped off was recorded. Ten ticks were tested per oil and

each filter paper strip was used only once. The attractant-treated filter paper was replaced with each new oil (every 10 ticks). The oils for which fewest ticks reached the tip and most dropped off – thyme, spearmint, ginger, geranium, turmeric, peppermint and lavender, were then retested at a concentration of 5%, alongside 20% DEET and 5% PMD after drying times of 1 and 4 h post-treatment to examine their residual activity. Turmeric was also retested at 2.5% and 1.25%.

2.2 Blanket-drag sampling

Following the results of the *in vitro* assay described above, turmeric oil was used as a repellent and orange oil was used as a hydrophobic negative control. Both oils were tested at 2.5 % (v/v) in a 1 % coco-glucoside excipient diluted in water. DEET (20% v/v) was used as a positive control and 1 % coco-glucoside diluted in water as an excipient-only control. Each treatment type was applied to four 1m² white cotton blankets (16 in total); treatments were placed in a pump-action spray bottle and each blanket was sprayed in a fume cupboard 100 times on each side, at one pump every 10 x 10 cm. Each pump delivered approximately 0.275 ml and the application rate delivered approximately 55 ml of treatment suspension per blanket. The blankets were then placed in individual airtight bags for transport to the field. Blankets were machine washed before being re-used and only blankets sprayed with the same treatment suspension were washed together.

The study site was at the edge of woodland within Ashton Court Estate, in south west England containing wild and managed deer populations and where ticks are abundant (Jennett et al., 2013). An area adjacent to the woodland edge was used for sampling. The area was divided into 16, 2 x 10 m sections, marked out using bamboo poles. Blanket-dragging took place in April 2016 at the same time on each of four successive days.

At the beginning of the trial, each investigator took one blanket out of its bag and attached it to a 1.3m long bamboo pole. The blankets were then placed flat on the ground at the start of their designated section and were dragged slowly in a straight line for 10 m, as described by Jaenson et al. (2006). After this, the blankets were immediately turned over and the number of ticks that had become attached to the underside were counted and recorded (initial tick count). The blankets were then turned back over so the ticks were on the underside and able to drop off. After one min the ticks remaining were re-counted (second tick count). The remaining ticks were removed. Only adult and nymphal ticks were counted.

Within the sample area, the section in which each blanket was dragged was randomised, until over the four-day sample period, all four treatments had been tested in all 16 sections.

2.3 Participatory *in vivo* study

The *in vivo* study used the same park location as the blanket drag trials. Dog owners were recruited by the researchers as they arrived to walk their dogs. The eligibility criteria for inclusion were that the dogs had previously had at least one tick infestation and that they would be walked in the park at least three times per week during the following four weeks. This ensured that each dog was exposed to a similar level of tick bite risk, as well as demonstrating that the owners had some awareness of ticks. Dog owners who reported that they did not know if their dog had ever had a tick were not included in the trial. No attempt was made to exclude dogs on the basis of recent acaricidal treatment, as based on previous work, it was considered that many owners were unable to provide an accurate report of the type or date of treatment (Jennett et al., 2013).

If all the eligibility criteria were met, the owners were given a consent forms and the nature of the study was explained. The dogs were then randomised into one of three groups; two treated and one untreated. Each owner was given a survey pack per dog, which included a tick diary, a tick removal tool and a pre-stamped and addressed envelope. Each tick diary contained brief aims of the study, some facts about ticks and the pathogens they can transmit, information on how to check for, identify, and remove a tick with the tool provided, as well as a short questionnaire about the dog. All owners were asked to continue to walk their dog as normal, check them for ticks on a daily basis and then fill in the corresponding day in the tick diary for 28 days; recording the date, where they walked (including walks outside of the study sites), whether or not they found any ticks on the dog and, if a tick was found, what body position of the dog it was on. The owners were asked to return their completed tick diaries using the envelope provided after 28 days.

The *in vivo* study used two essential oils: turmeric oil and orange oil. Both oils were used at a concentration of 2.5 % (v/v) diluted in water with a 1% coco glucoside excipient and mixed individually in 200 ml aluminium spray bottles; it was estimated that this amount was sufficient for at least four weeks of daily use for one dog. The orange oil acted as a negative control and allowed confirmation that any effects seen were not due to the hydrophobic nature of oils.

Owners of dogs allocated to the two oil-treatment groups were given a bottle of essential oil suspension and instructions on its application. They were asked to spray their dog at the beginning of each walk and a diagram was provided which highlighted the areas to be sprayed (legs and belly) and the amount to be applied relative to the size of the dog. A medium sized dog, for example, was to receive 10 sprays (two on each leg and two on the belly). Owners of dogs in the non-oil treatment group were asked to walk their dogs as normal and maintain the tick diary as for owners of dogs in the other two groups.

2.4 Statistical analysis

For the *in vitro* bioassay, Fisher's Exact Test was used to examine differences between the oils in the proportion of ticks that reached the top of the filter paper or that dropped off. For the blanket dragging study, the numbers of ticks counted were transformed ($\log_{10}(x+1)$) to normalise the variance and a one-way ANOVA with Tukey's post-hoc test used to test for differences between the initial tick counts of the four treatment groups. The log-transformed second tick count was compared to the initial tick count using a paired t-test and the percentage difference in tick count reduction was compared between groups using a Kruskal-Wallis non-parametric ANOVA using a Dunn-Bonferroni correction. For the *in vivo* trial, logistic regression was used to examine the association between trial group and the presence of ticks. For dogs with at least one tick, Chi-square tests of homogeneity were used to test for differences between trial groups in the number of dogs with ticks attached in total or the number of dogs with ticks in the sprayed areas (legs and belly). Post-hoc analyses involved pairwise comparisons using the z-test of two proportions with a Bonferroni correction, to identify significant differences between individual groups. Finally, the difference between groups in the total number of ticks acquired and the number of days on which ticks were found were analysed using one-way ANOVA and Tukey post-hoc tests. All data were analysed using SPSS (v.23, IBM).

3. Results

3.1 Repellency bioassay

The sebum extracted from the canine hair proved to be a strong attractant as, when it was present alone, 88% of ticks moved to the tip of the filter paper strip. When ethanol-only-

and ethanol + olive oil treatments were also applied to the top 1 cm of the vertical filter paper strip, in addition to the sebum, 85% and 82.5% of ticks reached the tip, respectively.

The percentage of ticks that reached the tip of the filter paper (Fig. 2) or dropped off (Fig. 3), was strongly affected by treatment ($P<0.001$). No ticks reached the tip of the filter paper strips when they were treated with spearmint, turmeric, thyme, geranium or ginger oils; no ticks reached the tip of the filter paper strip when it was treated with 20% DEET and 5% PMD. When orange, grapefruit, blackseed and soya were applied, similar numbers of questing ticks reached the filter paper tip as with the negative controls, indicating that these oils had no repellency. Fewer than 10% of ticks dropped off the negative control filter papers, whereas 70% dropped off both spearmint and thyme-treated filter papers. No ticks dropped off filter papers treated with PMD, while only 20% dropped off the DEET-treated filter papers.

After one hour of drying, there were significant differences in the number of ticks that reached the tip of the filter papers in the different treatment groups ($P<0.001$). DEET along with the oils of thyme and turmeric were the only treatments that continued to prevent all ticks from reaching the tip. Even after 4 hours, significantly fewer ticks reached the tips treated with turmeric, PMD or DEET compared to the negative control ($P<0.001$).

Turmeric was retested at 2.5% and 1.25% in order to determine its minimum effective concentration. No significant difference was observed between 5%, 2.5% or 1.25% concentrations in the number of ticks that dropped off the filter paper ($P=0.88$) and no ticks reached the tip at 5% and 2.5%. Hence, a concentration of 2.5% turmeric oil was selected for further field testing with orange oil as a control, since the latter appeared to have little effect.

3.2 Blanket-drag sampling

A total of 64 blanket-drags were performed during the trials. There were no major day-to-day differences in weather conditions (the mean ambient temperature was 11°C). In total, 899 ticks were counted, with an average of 23.3 (SD±21.3) ticks per blanket for excipient-only ($n = 16$), 26.9 (SD±28.6) for orange oil ($n = 16$), 2.6 (SD±2.0) for turmeric oil ($n = 16$) and 3.4 (SD±3.7) for DEET ($n = 16$). The difference in the numbers of attached ticks between the treatment groups was statistically significant ($F_{3,60} = 31.5$, $P<0.001$; Fig. 4). Tukey post hoc tests showed that the tick attachment rate was significantly lower for turmeric- and DEET-treated blankets than both the excipient-only ($P<0.001$) and orange-oil

($P<0.001$) treated blankets. There was no significant difference between turmeric and DEET ($P=0.95$) or excipient-only and orange oil ($P=1$) in the number of ticks attached.

Of the 899 ticks counted initially, 650 remained on the blankets after one minute. The mean of the second tick count (0.64, $SD\pm 0.61$) was significantly lower than the initial tick count (0.90, $SD\pm 0.49$; $t_{63} = 8.99$, $P<0.001$). The percentage decrease was significantly different across treatment groups (Kruskal-Wallis $H = 46.77$, $P<0.001$), with a mean decrease of 20.53% ($SE \pm 3.12$) for excipient-only, 19.56% ($SE\pm 3.32$) for orange oil, 93.03% ($SE\pm 4.15$) for turmeric oil and 89.42% ($SE\pm 3.92$) for DEET (Fig. 5). Pairwise comparisons showed that the percentage of ticks that dropped off the blankets after one minute differed significantly between excipient-only and DEET (Dunn-Bonferroni, $P<0.001$), excipient-only and turmeric oil ($P<0.001$), orange oil and DEET ($P<0.0001$) and orange oil and turmeric oil ($P<0.001$), but not between excipient-only and orange oil ($P>0.05$) or turmeric oil and DEET ($P>0.05$).

3.3 Participatory in vivo study

A total of 90 dogs were recruited (30 in each trial group) over a four-week period in May 2016. Owners who had not returned their tick diaries by the end of June were contacted by email or phone. There was an overall response rate of 61% (55 completed diaries) with 24, 16 and 15 completed in the turmeric oil, orange oil, and control groups, respectively. Seven owners reported that they could not complete the trial, mainly due to illness or holiday; 28 owners did not respond.

Thirty-nine dogs (71% $\pm 12.0\%$) were reported to have picked up at least one tick during the trial: 86% ($\pm 17.6\%$) of the control group, 81% ($\pm 19.2\%$) of the orange-treated group and 54% ($\pm 20.0\%$) of the turmeric-treated group (Fig. 6). Of the 39 dogs that had ticks during the trial, 37 (94% $\pm 7.4\%$) had at least one tick that was attached to the skin; two dogs had ticks that were found crawling on the fur only. Overall, logistic regression showed that there was no difference in the likelihood of dogs in the three trial groups acquiring a tick ($P=0.07$). However, interrogation of the diaries maintained by owners, showed that one dog gained a tick on a day it swam in a stream, two owners found ticks on days when they forgot to spray their dogs and three other ticks found on the first trial day were reported to be fully engorged and, therefore, were likely to have attached prior to starting the trial. If these instances are removed from the analysis, logistic regression shows that the dogs in the

turmeric oil-treated group were significantly less likely to have a tick than the other treatment groups ($B = -2.297$, df_2 , $P=0.014$).

Furthermore, the percentage of dogs with ticks attached in the sprayed body areas (legs and belly) also differed significantly between trial groups ($\chi^2 = 14.3$, df_2 , $P<0.001$; Fig. 7). The percentage of dogs with ticks attached to the legs or belly of dogs sprayed with turmeric oil suspension ($15\% \pm 19.4\%$) was significantly lower than that of ticks attached to the same areas of dogs sprayed with orange oil suspension ($85\% \pm 19.4\%$) and unsprayed dogs ($73\% \pm 26.2\%$) ($P<0.05$). There was no significant difference between the latter two groups ($P>0.05$).

In terms of tick numbers, 334 ticks were counted on dogs during the trial, with an average of 5.9 ticks on dogs in the control group ($SD \pm 5.5$), 9.8 ticks on dogs in the orange oil-treated group ($SD \pm 16.7$), and 3.7 ticks in dogs in the turmeric oil-treated group ($SD \pm 8.3$). The \log_{10} transformed tick counts were significantly different between groups ($F_{2,51} = 4.97$, $P<0.01$) and post-hoc tests showed that dogs sprayed with turmeric oil had significantly fewer ticks than dogs sprayed with orange oil ($P=0.02$) or dogs in the untreated control group ($P=0.04$). Dogs in the turmeric treatment group had ticks on significantly fewer days than dogs in the other two groups ($F_{2,51} = 4.35$, $P=0.02$).

4. Discussion

Arthropod repellents can be defined as chemical substances that cause arthropods to make oriented movements away from their source (Dethier et al., 1960). While this definition is easily applied to flying insects such as mosquitoes, this is not the case for crawling arthropods such as ticks. Ticks have a long-lasting host-parasite association and the term ‘repellency’ commonly subsumes a range of effects, including avoiding or leaving the host, failing to attach, to bite, or to feed (Halos et al., 2012).

The bioassay used here allowed different compounds and formulations to be compared under standardised laboratory conditions. The main characteristic of tick inappetence behaviours is an exhibition of negative geotaxis (MacLeod, 1935; Lees and Milne, 1951), therefore, the ability of an essential oil to abolish the climbing behaviour of a tick is an indicator of the degree of its repellency (Lwande et al., 1999; Birkett et al., 2011; Lima et al., 2016). Some of the essential oils tested here demonstrated a very strong repellence effect, as the majority of ticks exhibited positive geotaxis and either remained in

the lower part of the filter paper for the duration of the test, or dropped off the filter paper without making contact with the tip. This supports findings by McMahon et al. (2003), which suggest that more volatile compounds have a greater effect on a tick's olfactory system than others. Nevertheless, this effect reduced quickly over time, implying that there is a negative correlation between volatility and residual activity. In contrast, turmeric oil matched the efficacy and duration of 20% DEET at concentrations as low as 2.5% throughout both the laboratory and blanket-drag trials.

The main component of turmeric essential oil, turmerone, has a considerably lower vapour pressure than thymol and carvone which are the major components of thyme and spearmint oils respectively (Chowdhury et al., 2008). This may explain the marked differences in residual activity of the oils. Jaenson et al. (2006) used a similar blanket-dragging method to test the efficacy of MyggA Natural, a commercially available repellent in Sweden, which contains approximately 15% PMD and small amounts of geranium, lavender and rose extracts. It was 74% repellent against nymphal *I. ricinus* in the field. Using the approach described by Jaenson et al. (2006), the 2.5% turmeric suspension used in the present study was 90% effective during blanket-drag sampling, suggesting it was more repellent than both MyggA Natural in Jaenson's study and 20% DEET, which was 86% effective in the present study. These registered repellents are at least 8 and 12 times more concentrated than the concentration of turmeric used here. The activity of turmeric oil has previously been reported against other arthropods including the cockroach, *Periplaneta americana* (Ahmad et al., 1995), the rice weevil, *Sitophilus zeamais* (Ishii et al., 2010) and mosquitoes (Tawatsin et al., 2006).

Dogs sprayed with the turmeric essential oil suspension were less likely to have a tick attach or feed during the trial compared to dogs in the negative control and untreated control groups; the difference was close to significance ($P=0.061$) and exceeded significance when confounding factors, such as owner compliance, were accounted for. Notably, in this trial, the dogs were only sprayed on the legs and belly, so the majority of the animal was unsprayed and there was a highly significant difference in the percentage of dogs acquiring ticks in the sprayed areas of the body in the different treatment groups. Only two dogs in the turmeric oil-treated group were found to have ticks on their legs or belly, which was significantly fewer than the placebo and control groups. This supports the conclusion that the turmeric suspension caused contact repellency.

The current field trial relied entirely on owner compliance, which has been highlighted as a concern in relation to monthly ectoparasite control retreatment recommendations (Beck et al., 2013). This might have been reflected in the 61% response rate, with people not willing, forgetting, or not having time to apply a daily product. Other people might have started the trial but stopped when observing no benefit, which might explain why the response rate for the control and orange-oil treatment groups were lower than that of the turmeric-oil treatment group. It is likely that the owners who did respond were those with the greatest interest in ticks, natural treatment alternatives and/or a history of tick problems, which could have affected the outcome.

In the type of trial undertaken here, it is extremely difficult to distinguish repellency ‘*sensu stricto*’ from expellency (ticks that initially attached then detached). Distinctions between those factors are important, particularly in preventing tick-borne pathogen transmission. Here, it was not recorded how long after each walk that owners checked for ticks. It is likely that some owners checked their dogs immediately after each walk, which might have resulted in them finding ticks that otherwise would have dropped off a few hours later. Neither a feeding nor killing effect was measured, as owners were advised to remove and dispose of any detected ticks immediately. A few owners reported finding ticks in their dog’s bed; however, it was not known whether they had dropped off within 24 h, whether it was due to the essential oil spray, routine acaricidal treatment, or another reason, or if they were fully engorged and had been attached and undetected for a few days.

The results suggest that even at a low concentration the turmeric essential oil suspension could be used as a repellent for dogs, whose owners do not want to use conventional synthetic chemical products and who are willing to undertake repeated applications; 2.5% turmeric essential oil demonstrated repellency comparable to 20% DEET and was more effective than 5% PMD *in vitro*. The data suggest that the spray has the potential to significantly reduce the number of tick bites, and when combined with avoidance of tick-infested habitats and thorough examination after visiting these areas, it could form part of an effective integrated control strategy to decrease the risk of tick-borne disease, either alone or in combination with other approaches. The low toxicity and natural ingredients may make the essential oil suspension appealing to dog owners particularly if the challenges of low persistence and residual activity can be overcome. Further detailed field testing however is required to understand their mode of action and identify optimum formulations and application strategies (Ellse and Wall, 2013).

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Figures

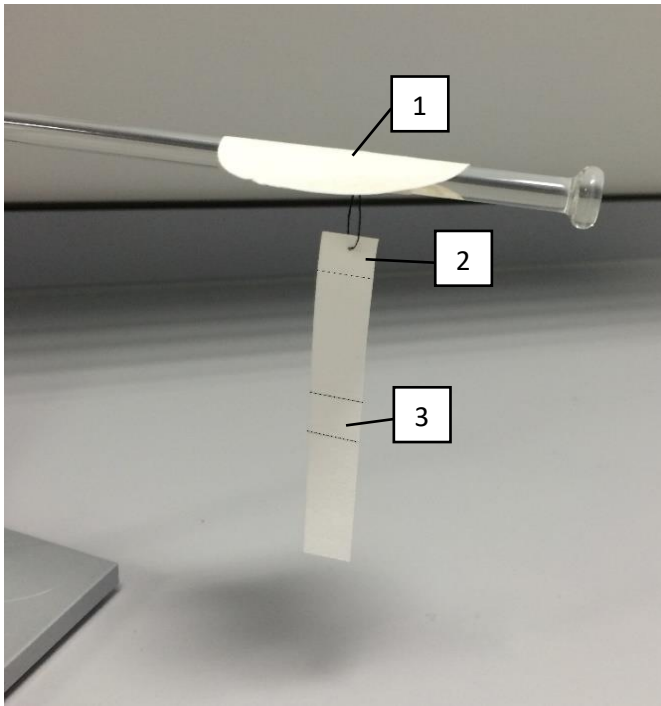


Fig. 1. The repellency bioassay apparatus showing 1) the attractant, 2) the treated-tip and 3) the starting point.

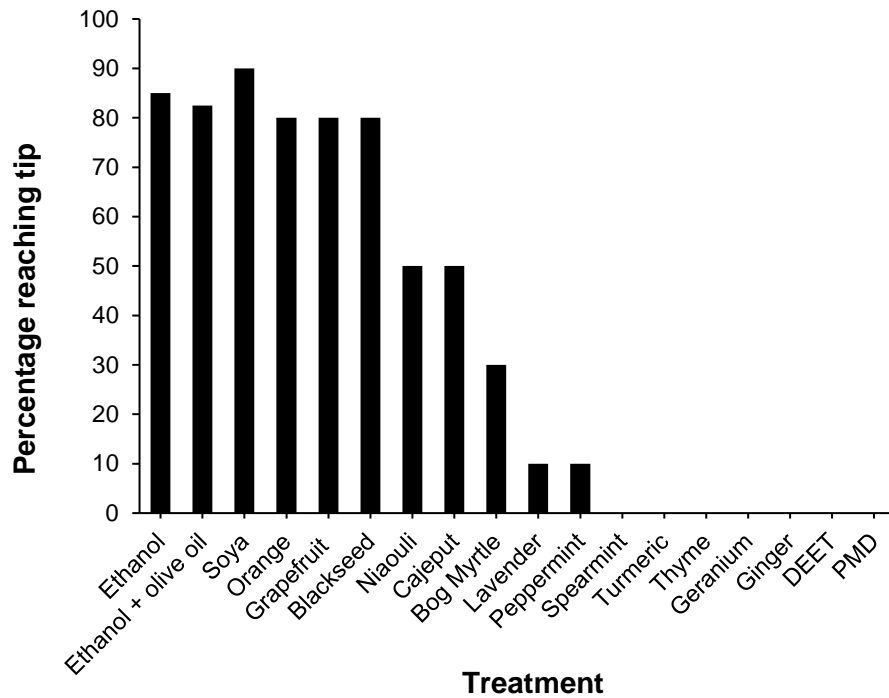


Fig. 2. The percentage of ticks (*I. ricinus*) that reached the tip of filter paper treated with 5% (v/v) solutions of oil (20% for DEET).

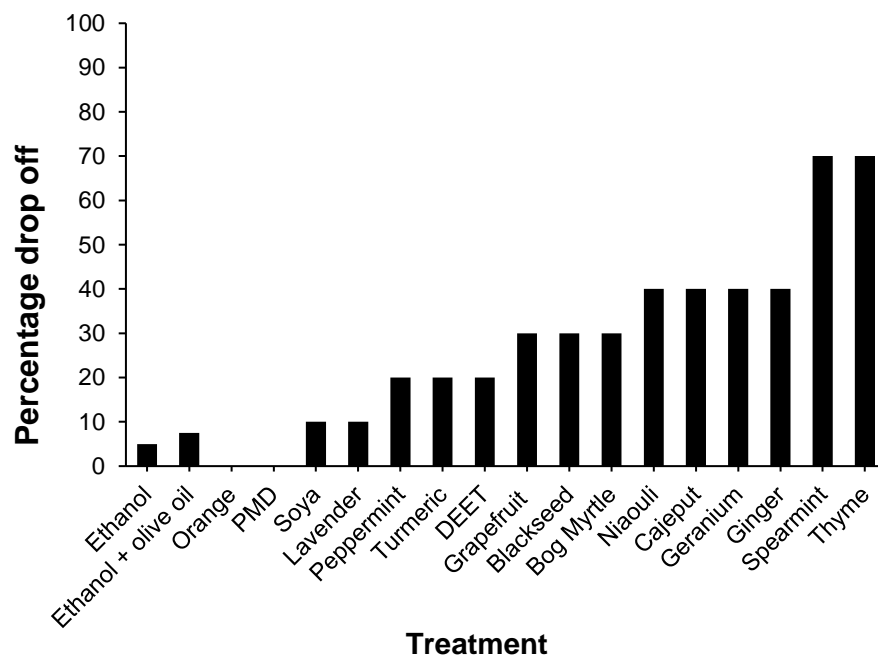


Fig. 3. The percentage of ticks (*I. ricinus*) that dropped off filter paper treated with 5% (v/v) solutions of oil (20% for DEET).

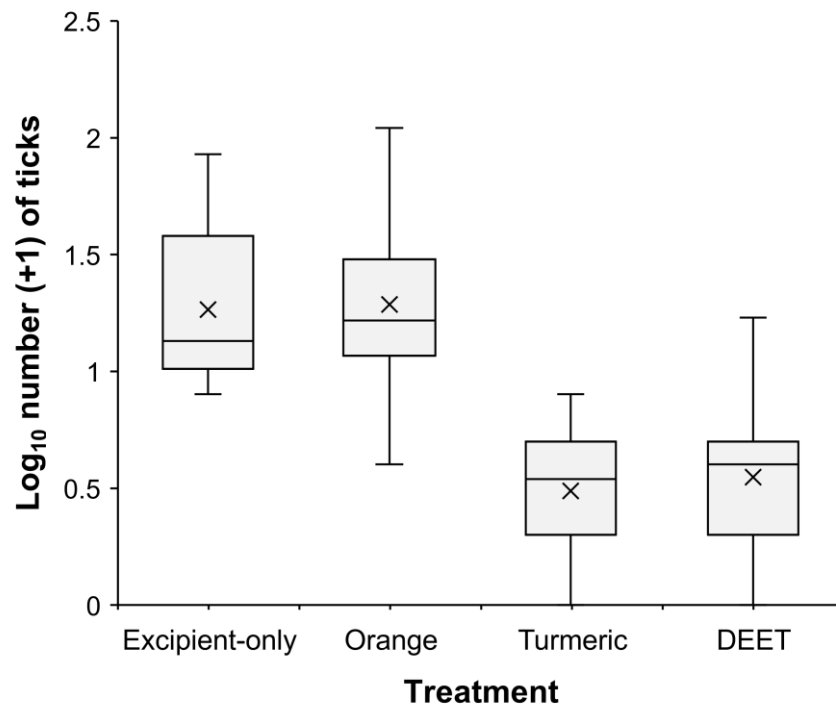


Fig. 4. The initial number of ticks (*I. ricinus*) counted on blankets treated with excipient-only (n = 16), 2.5% orange (n = 16), 2.5% turmeric (n = 16) and 20% DEET (n = 16). Box plot with mean (crosses), median (horizontal bar), 1st and 3rd quartile (box) and the minimum and maximum value (capped bars) of the log₁₀ transformed data.

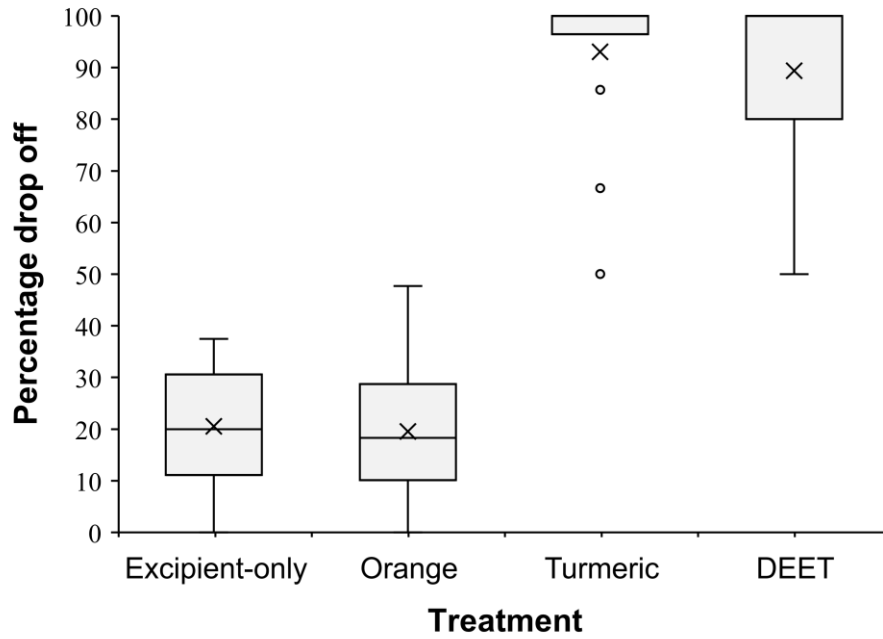


Fig. 5. The percentage of ticks (*I. ricinus*) that dropped off blankets treated with excipient-only (n = 16), 2.5% orange (n = 16), 2.5% turmeric (n = 14) and 20% DEET (n = 15), within one minute. Box plot with mean (crosses), median (horizontal bar), 1st and 3rd quartile (box), interquartile range (capped bars) and outliers (dots).

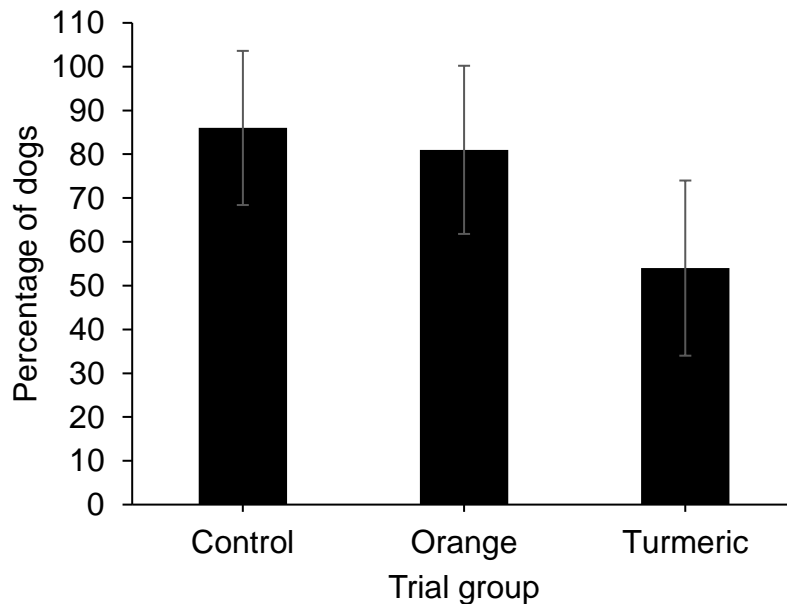


Fig. 6. The percentage of dogs (± binomial 95% confidence intervals) in each trial group that had at least one tick during the trial.

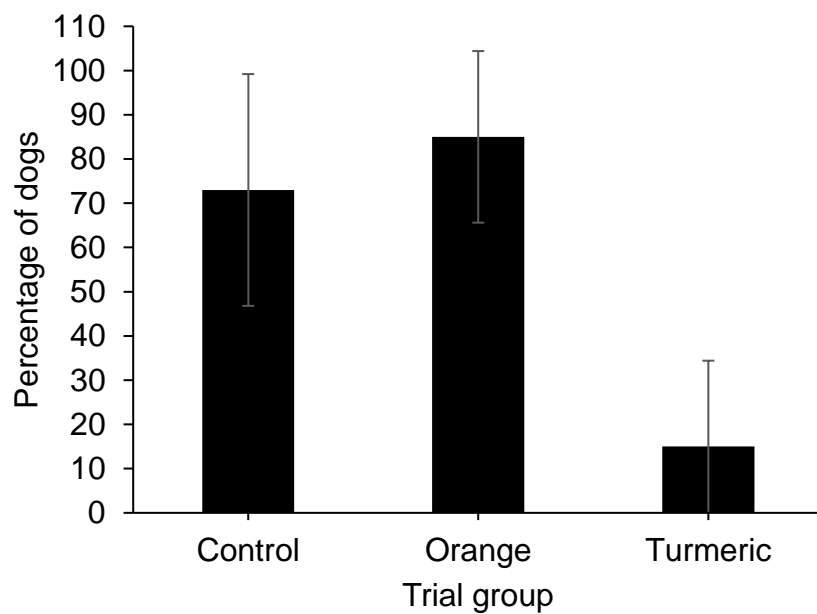


Fig. 7: The percentage of the dogs (\pm binomial 95% confidence intervals) that acquired at least one tick, that had the tick attached to their legs or belly.